

## **Remarks**

### **Rejections under 35 U.S.C. § 112**

Claims 25, 26, and 31 stand rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. While disagreeing with the Examiner's position with respect to this issue, in the interests of furthering prosecution at this time, the claims have been cancelled without prejudice to the pursuit of some or all of them in the future.

### **Rejections under 35 U.S.C. § 103**

Claims 1-3, 5-8, 19 and 21-32 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Holmes et al. (5,955,343), hereinafter "Holmes" in view of Hubbell (6,129,761), hereinafter "Hubbell". Applicants respectfully traverse this rejection for each of the reasons set forth below.

The Examiner asserts that, "The rejection is not based on Hubbell et al suggesting forming a hydrogel, but on Hubbell et al suggesting encapsulating cells in the membrane or matrix of Holmes et al rather than attaching cells to the membrane or matrix." However, Applicants submit that Hubbell does not suggest encapsulating cells in the membrane or matrix of Holmes. Holmes discloses membranes comprised of short, self-assembling peptides having sequences not found in mammalian organisms. Hubbell teaches the use of implantable, cell-containing scaffolds made of any of numerous polymers including carbohydrates such as alginate and various polysaccharides. Hubbell briefly mentions that, "Other materials which may be utilized include proteins such as fibrin, collagen, and gelatin." (col. 8, lines 61-62). This teaching does not suggest the use of short, non-naturally occurring peptides for encapsulation of cells since, among other reasons, all the examples of proteins provided by Hubbell are large proteins that naturally occur within the body of mammalian organisms. Hubbell's extensive teachings regarding the utility of carbohydrate-based structures and his minimal discussion of large, naturally occurring proteins for encapsulating cells does not suggest that cells could be successfully encapsulated in materials with very different compositions and properties such as structures composed of the self-assembling peptides of Holmes.

Since Hubbell presents no data or working examples that actually demonstrate encapsulation of cells in any of the numerous materials suggested for use in the practice of his methods, one of ordinary skill in the art would conclude that Hubbell had merely surveyed the literature and identified as many materials as possible whose properties he deemed *potentially* suitable for the production of his scaffolds. EAK-16, the peptide most extensively discussed in Holmes, was described in the prominent scientific journal *Proceedings of the National Academy of Sciences* in April 1993, well before the June 1995 filing date of Hubbell's application. See Exhibit A, which is a copy of the article (authored by several of the inventors listed on the Holmes patent) reporting on the EAK-16 peptide and its properties, including its ability to form membranes. One of ordinary skill in the art would have expected Hubbell, as a skilled artisan in the area of hydrogel materials, to be familiar with this work. The fact that Hubbell notably fails to mention the possibility of using short, synthetic, self-assembling peptides such as EAK-16 in the practice of his methods, while mentioning a large and diverse set of other materials, would lead one of ordinary skill in the art to conclude that Hubbell had considered such peptides, and materials formed from them, unsuitable for his purposes.

Indeed Hubbell teaches away from the concept of encapsulating cells in the membrane of Holmes. Hubbell teaches the use of "slowly polymerizing" hydrogels (see Abstract and Summary of the Invention, col. 5, ~ line 6). Holmes, however, teaches that, "formation of the membranes is very fast" (col. 8, line 21). While it is recognized that terms such as "slow" and "fast" are relative, it is significant that Hubbell chooses to emphasize slow polymerization as important while Holmes characterizes the self-assembly process as fast, leading one of ordinary skill in the art to conclude that the peptides of Holmes are unsuitable for encapsulation of cells as taught by Hubbell.

In addition to the fact that Hubbell does not teach or suggest the encapsulation of cells in the membrane of Holmes, as discussed in the previous office action response, the combination of Holmes and Hubbell does not enable the instant invention. There is no teaching in Hubbell or in Holmes of the proper way to combine the references so as to achieve the intended useful result disclosed by Hubbell, i.e., a cell-containing scaffold suitable for implantation into a subject for therapeutic purposes. Developing the method to encapsulate cells in a macroscopic scaffold

formed by peptide self-assembly, as described in the present application and recited in the claims, required additional inventive steps not disclosed or suggested by Holmes or Hubbell.

Holmes discloses a method in which peptides are added to tissue culture medium of cultured cells, which resulted in the formation of membranes that did not encapsulate the cells (see, e.g., col. 3, lines 32-35 of Holmes). Holmes further discloses that various monovalent cations can induce membrane formation (see, e.g., col. 7, lines 50-60). Holmes discloses that cells *attach to* the membranes and that they are useful for culturing cell monolayers on the surface of the membranes (see, e.g., col. 11, lines 32-43). Holmes does not discuss how these teachings might be modified to successfully *encapsulate* living cells. Such teachings are to be found only within the instant application. In particular, as described on page 2, lines 13-15, of the instant application, a preferred method of producing a scaffold encapsulating cells “involves incubating peptides and living cells in an aqueous solution having an iso-osmotic solute, preferably under conditions that do not allow the peptides to substantially self-assemble.” This step is followed by addition of an electrolyte, which allows self-assembly to proceed. (See p. 2, lines 18-21). See also p. 15, lines 1-5, stating, “We have discovered that a peptide scaffold that encapsulates living cells in a three-dimensional arrangement may be formed by first mixing the cells and the peptides in a solution having the required osmolarity to maintain cell viability, and then adding sufficient electrolytes to initiate self-assembly of the scaffold.” For example, the scaffold encapsulating cells may be formed by dissolving peptides in an iso-osmotic solution in the absence of electrolyte, resuspending cells in the solution, introducing the solution into a casting frame or mold, and then exposing the solution to a sufficient concentration of electrolyte to allow self-assembly to occur (p. 23, line 24 – p. 24, line 6).

The Examiner has stated that Holmes suggests this method by disclosing that the peptides do not self-assemble in aqueous solution until a salt is added. Applicants contend, however, that this method and the results achieved thereby are only obvious in hindsight, i.e., once the teachings of the instant application have been appreciated. Based on the teachings of Holmes, one of ordinary skill in the art would have no reason to conclude that exposure of a solution containing dissolved peptides and cells to salt would result in formation of a three-dimensional scaffold in which the cells would be contained, as required by the instant claims, rather than formation of thin, flat membranes that would likely exclude the cells. Such membrane formation

occurred according to the teachings of Holmes when a peptide-containing solution was added to a solution containing a salt (col. 3, lines 21-25, lines 45-51, lines 57-62; col. 14, lines 48-54).

Furthermore, the fact that the peptides do not self-assemble in aqueous solution until a salt is added does not suggest maintaining cells and peptides *in an iso-osmotic solution* substantially free of electrolyte prior to addition of an electrolyte in sufficient concentration to cause self-assembly. Holmes nowhere suggests such a method. It was Applicants' recognition that in order to produce a scaffold comprised of self-assembling peptides that encapsulates cells as required by the instant claims, the peptides should be dissolved under conditions under which self-assembly cannot occur and combined with cells under conditions in which self-assembly also cannot occur until the cells are distributed appropriately in the solution. However, in order for the cells to remain in a viable state, they cannot be maintained for extended periods of time under non-iso-osmotic conditions. Thus Applicants conceived of the idea of maintaining the cells in an iso-osmotic solution substantially free of electrolytes prior to combination with a peptide solution or, alternatively, dissolving the peptides in an iso-osmotic solution substantially free of electrolytes and then adding cells (see, e.g., p. 23, lines 24-25, describing that the peptides were dissolved in a solution containing an iso-osmotic solute, i.e., 295 mM sucrose, prior to addition of cells). Only after the peptides and cells are mixed is scaffold formation allowed to occur. Holmes does not suggest this approach and thus cannot enable successful encapsulation of living cells within a scaffold comprised of the self-assembling peptides taught therein. Applicants further note that it was not obvious that self-assembly would proceed even in the presence of the sucrose, i.e., that the presence of substantial concentrations of sucrose would not interfere with gel formation.

Similarly, Hubbell does not suggest a method that could successfully be used to encapsulate cells in scaffold comprised of self-assembling peptides. In describing how to produce a hydrogel encapsulating cells, Hubbell states that, "Preferably the polymer is dissolved in an aqueous solution, preferably a 0.1 M potassium phosphate solution, at physiological pH, to a concentration forming a polymeric hydrogel...The isolated cells are suspended in the polymer solution..." (col. 10, lines 31-33). Simply reading this cursory description would not apprise one of ordinary skill in the art of the method of dissolving the peptides in a solution lacking electrolyte, maintaining the peptides and living cells together *in an iso-osmotic solution* under

conditions that do not allow the peptides to substantially self-assemble, and then exposing the solution to an electrolyte to initiate self-assembly. It is evident that were one to follow the teachings of Hubbell in an attempt to encapsulate cells using self-assembling peptides, one would suspend the peptides in an aqueous solution under conditions (e.g., 0.1 M potassium phosphate) *under which self-assembly may actually begin* and then attempt to suspend cells in the mixture. Applicants submit that one of ordinary skill in the art would consider it more likely that adding cells to a solution in which peptide self-assembly has already started to occur would result in exclusion of cells from any self-assembled structure that might actually form rather than encapsulation. In fact, the inventors discovered that the peptides do not appear to dissolve when suspended in salt-containing solutions at physiological pH as taught by Hubbell but rather remain in a particulate-appearing form. Thus any self-assembly that occurs would be minimal and on a microscopic scale and would not result in formation of a macroscopic scaffold as required by the instant claims.

Alternately, if one were to follow the teachings of Hubbell but with the recognition that it would be desirable to avoid exposing the peptide solution to electrolyte before thoroughly distributing the cells therein, one would dissolve the peptides in an aqueous solution as suggested by Hubbell, but would select a solution lacking a significant concentration of electrolytes. However, if one were to suspend cells in such a solution as taught by Hubbell without ensuring that the cells were maintained under iso-osmotic conditions, the result would be lysis of the cells. While Hubbell teaches that hydrogel precursor solutions can be “osmotically adjusted” with a nonion, this is mentioned in connection with injection of polymer/cell suspensions into a subject. One of ordinary skill would recognize that the purpose of such osmotic adjustment is to minimize the shock to the body associated with injection of a solution having a different osmolality to that existing *in vivo*. Such a vague reference, lacking any detailed guidance, would not by any means apprise the skilled artisan of the methods necessary to produce a scaffold encapsulating cells using the self-assembling peptides of Holmes. It was Applicants’ experimental work that provided the enabling steps in the methods for successful encapsulation of cells in a scaffold comprised of self-assembling peptides.

As the Federal Circuit has repeatedly affirmed, “ ‘In order to render a claimed apparatus or method obvious, the prior art must enable one skilled in the art to make and use the apparatus

or method.’ ” *Motorola, Inc. v. Interdigital Technology Corp.*, 121 F.3d 1461 (Fed. Cir. 1997), quoting from *Beckman Instruments, Inc. v. LKB Produktur AB*, 892 F.2d 1547 (Fed. Cir. 1989). Furthermore, “In holding an invention obvious in view of a combination of references, there must be some suggestion, motivation, or teaching in the prior art that would have led a person of ordinary skill in the art to select the references and combine them *in the way that would produce the claimed invention*.” *Karsten Manufacturing Corp. v. Cleveland Golf Co.*, 242 F.3d 1376 (Fed. Cir. 2000) (emphasis added). Even if the teachings of Hubbell could be said to suggest the use of self-assembling peptides to form hydrogels encapsulating viable cells, neither Holmes, Hubbell, or a combination of both discloses how to actually achieve this result. In particular, these references do not suggest the importance of maintaining the cells and peptides under iso-osmotic conditions prior to exposure to the electrolyte.

In addition, even if the combination of Holmes and Hubbell suggested and enabled the use of the self-assembling peptides of Holmes to encapsulate cells in a three-dimensional arrangement, the skilled artisan would have had no reasonable expectation that the cells would survive once encapsulated. It is noted that the instant claims recite “living cells”. Holmes presents data showing that neurons can attach to the surface of a two-dimensional membrane formed by self-assembly of amphiphilic peptides and that the membranes are not toxic to these cells (col. 10, lines 45-50). However, the mere fact that cells can grow on the surface of a membrane formed by self-assembly of amphiphilic peptides is not sufficient to provide a reasonable expectation that these cells, or any cells, would be able to survive under the very different conditions of encapsulation *within* a scaffold formed by self-assembly of the peptides. Firstly, Holmes provide no evidence to suggest that cells could survive the rapid changes in the extracellular environment associated with peptide self-assembly, in which cells go from a state in which they are in solution to one in which they are constrained in three dimensions. Second, when cells are grown on a membrane, only one surface of the cell is in contact with the hydrogel material, which allows the upper surface of the cell to remain unconstrained and limits the area in contact with the material to at most 50% of the total cell surface. In contrast, since cells are generally much larger than the pore size of the membrane, encapsulation would result in a situation in which the cells are contained in all dimensions (col. 12, line 6-9). Holmes suggests that the structure of the membranes resembles that of the neurofibrillary tangles and amyloid

plaques associated with neuropathological conditions such as Alzheimer's disease (col. 12, lines 31-40). One of ordinary skill in the art might well be expected to doubt whether cells would survive when confined within a material with such a structure.

Until Applicants successfully encapsulated cells and measured their survival within the scaffold (see page 24, lines 15-20), there was no reason to believe that such survival could be achieved. Indeed only 75% of the cells did survive after 24 hours, further indicating that it was not obvious that cells would survive encapsulation in self-assembling peptide hydrogels. Thus one of ordinary skill in the art, reading Hubbell and Holmes, would not be provided with a reasonable expectation either that cells *could be* encapsulated in scaffolds formed by self-assembly of amphiphilic peptides *or* that cells so encapsulated would survive to form a structure useful for implantation. As the Federal Circuit has stated, "'For a [prior art reference] to render the claimed invention obvious, there must have been, at the time the invention was made, a reasonable expectation of success..." *Life Technologies, Inc. v. Clontech Laboratories, Inc.*, 224 F.3d 1320 (Fed. Cir. 2000), citing *Micro Chem., Inc. v. Great Plains Chem. Co.*, 103 F.3d 1538, 1547 (Fed. Cir. 1997) and *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988). At the very most, one of ordinary skill in the art, reading the cited references would have found it obvious to try to encapsulate cells in such a material. However, the Federal Circuit has consistently stated that, "'[O]bvious to try' is not the standard." *Eclochem, Inc. v. Southern California Edison Co.*, 227 F.3d 1361 (Fed. Cir. 2000).

In addition to the fact that the combination of Hubbell and Hubbell does not teach or enable the instantly claimed invention, a number of secondary considerations support a determination of non-obviousness. As mentioned above, the inventors of Holmes disclosed self-assembling peptides and membranes formed therefrom in 1993. A PCT application (WO9640304, entitled "Injectable Hydrogel Compositions", included herein as Exhibit B) virtually identical to Hubbell's U.S. patent was published in December 1996. Given the intense research activity in the field of tissue engineering during the 1990s, had the teachings of Hubbell indeed rendered obvious the successful encapsulation of cells in the membrane of Holmes, one might have expected such encapsulation to have been rapidly reported in the patent and/or scientific literature soon after the teachings of Hubbell entered the public domain. However, the first report of encapsulation of cells in scaffolds comprised of the self-assembling peptides of

Holmes only occurred more than three years later, with publication of an abstract entitled “A New Self-Assembling Peptide Gel for Cartilage Tissue Engineering: Chondrocyte Encapsulation and Matrix Production” (International Cartilage Repair Society, 3<sup>rd</sup> Symposium, Gothenberg, Sweden, April 27 - 29, (2000)), which lists the inventors of the instant application as authors and describes certain aspects of the instantly claimed invention. This and additional work describing the formation of self-assembling peptide scaffolds encapsulating cells was subsequently published in the *Proceedings of the National Academy of Sciences* (see Exhibit C). Applicants submit that it is unlikely that the invention would have merited presentation at an international scientific meeting and publication in a prestigious scientific journal if it was merely an obvious extension of teachings that had been in the public domain for over three years.

As further evidence of the non-obviousness of the instantly claimed invention, the Examiner’s attention is directed to Exhibit D, which is a recently published review article authored by Jeffrey A. Hubbell (i.e., the inventor of the Hubbell patent). The article discusses various materials for use in tissue engineering that would mimic properties of the three-dimensional extracellular matrix (ECM) in which cells exist in native tissues (see p. 551, first paragraph). The article describes a variety of materials, including gel matrices such as those listed in Hubbell’s patent (see, e.g., page 553 of the article, which includes an extensive description of alginate, one of Hubbell’s preferred materials). Hubbell references the inventors’ paper in the *Proceedings of the National Academy of Sciences* (2000) (Exhibit B) and rates the work described therein as “of outstanding interest” (see p. 555, right-hand column, line 7, in which reference 91 is given two circles, and see the key under “References and recommended reading” on p. 555, stating that two circles indicates papers of outstanding interest). Applicants submit that it is unlikely that Hubbell, a recognized expert in the field of materials for tissue engineering, would have conferred this praise on a paper describing the instant invention had he considered it an obvious variation on what is described in his own previously issued patent. As the Federal Circuit has recently recognized, “Appreciation by contemporaries skilled in the field of the invention is a useful indicator of whether the invention would have been obvious to such persons at the time it was made.” *Vulcan Engineering Co., Inc. v. Fata Aluminum, Inc.*, 278 F.3d 1366 (Fed. Cir. 2002).



Not only does Hubbell praise the instant invention, he also recognizes that it addresses a long felt but unsolved need in the field of tissue engineering, namely the need for an ECM-like material having both tensile fibrillar elements and compressive gel elements (see Exhibit D, p. 555, upper right column). The fact that three-dimensional scaffolds formed from the peptides and encapsulating cells would exhibit this desirable combination of properties is nowhere suggested by either Holmes or Hubbell. Providing a solution to a long felt but unsolved need is one of the three key secondary indicators of non-obviousness recognized by the Supreme Court (see *Graham v. John Deere Co.*, 383 U.S. 1, 17 (1966)).

In summary, the combination of Holmes and Hubbell does not render the invention of claim 1 obvious because (i) the combination does not teach the invention without the use of hindsight; (ii) the characteristics Hubbell describes as desirable for materials useful to practice his invention (e.g., slowly polymerizing, carbohydrate or polysaccharide-based) contrast with the properties and terms Holmes uses to characterize the membranes and their formation (e.g., rapid formation, made of short synthetic peptides not found naturally in mammals) (iii) additional inventive steps found only in the instant application are necessary to enable the claimed invention; and (iv) the combination of references does not provide a reasonable expectation of success in achieving a useful result.

Furthermore, secondary considerations strongly support a finding of non-obviousness, and the Federal Circuit has repeatedly recognized that “The secondary considerations are...essential components of the obviousness determination” *In re Rouffet*, 149 F.3d 1350 (Fed. Cir. 1998). These considerations include (i) the availability of the inventors’ 1993 *PNAS* publication describing membranes comprised of self-assembling peptides in 1993, and the availability of Hubbell’s PCT application in 1996 in the public domain well before the filing of the instant application; (ii) praise of others in the field, as evidenced by the fact that Hubbell, an expert in the field, considered Applicants’ scientific article describing certain aspects of the instant invention as of outstanding interest; and (iii) solution of a long felt but unsolved need, as evidenced by the recognition that the instantly claimed invention addresses the need for materials that have both tensile fibrillar elements and compressive gel elements for tissue engineering. Withdrawal of the rejection is respectfully requested.

Given that claim 1 is non-obvious, Applicants submit that claims 2, 3, 5-8, 19 and 21-32, which depend on claim 1, are also non-obvious. In addition, Applicants submit that certain of these claims are also non-obvious for a variety of other reasons. With respect to claims in which chondrocytes are encapsulated (e.g., claim 19), the Examiner has stated that since Holmes suggests that the peptides can be combined with collagen (col. 11, line 58), it would have been obvious to use chondrocytes to produce the collagen. Applicants submit that based on a fair reading of Holmes it is clear that Holmes envisions a process in which peptides and collagen are combined, rather than a process in which encapsulated cells secrete collagen. The collagen would have been produced using any of various well-known methods for obtaining collagen for use in tissue engineering, e.g., by harvesting the material from animal tissue. The Examiner's suggestion that it would have been obvious to encapsulate chondrocytes to provide a source of the collagen is based entirely on hindsight.

The Examiner has also stated that compression as required by claim 30 would not result in a different membrane or matrix than that obtained by Holmes and that handling the membrane of Holmes would inherently result in some compression. Applicants respectfully disagree. Firstly, it is clear that any incidental handling to which the membranes of Holmes may be subjected does not fall within the meaning of "compression" as described in the instant specification and understood by one of ordinary skill in the art. As described in the instant specification, "compression" does not refer to minor physical contact occurring in a random pattern but rather to a significant, preferably predetermined, well-defined and repeatable, application of force. For example, "A preferred compression scheme includes dynamic compression at 0.01 to 3 Hz or more preferably 0.1 to 1 Hz, superimposed on a static offset compression. Typically, the dynamic strain amplitude is between 0.01 and 10%, preferably, between 1 and 5%, and, more preferably, between 3 and 5%, and the static offset compression is between 5 and 15%" (p. 8, lines 11-19). Given that the claims must be read in light of the specification, it is unreasonable for the Examiner to equate compression, as used in claim 30, with incidental physical contact during handling. Secondly, in contrast to the assertion of the Examiner, in accordance with the teachings of the instant invention, compression does result in a different matrix to that obtained by Holmes. As taught in the instant application, "Preferably, the compression scheme increases the equilibrium compression modulus of the macroscopic scaffold

by at least 5, 10, 20, 30, 50, 100, 200, 300, 400, or 500 kPA or by at least 2, 5, 25, 50, 75, or 100-fold compared to a control macroscopic scaffold not subjected to the compression scheme. In another preferred embodiment, the compression scheme induces the secretion of extracellular matrix components by the cells.” (p. 8, lines 19-24). In particular, compression causes encapsulated cells to increase their secretion of extracellular matrix components relative to the level of synthesis that would occur in the absence of compression.

Claim 4 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Holmes and Hubbell as applied to claims 1-3, 5-8, 19, and 21-32 and further in view of Holmes et al. (PNAS). Given that claim 1 is non-obvious, Applicants submit that claim 4, which depends on claim 1, is also non-obvious.

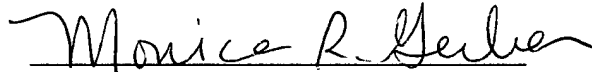
Claim 20 stands objected to as being dependent on a rejected claim, namely claim 1. Applicants submit that since claim 1 is non-obvious, claim 20 is allowable.

In conclusion, in view of the amendments and remarks presented herein, none of the cited art anticipates any of the claims pending in the instant application nor renders them obvious. Applicants therefore respectfully submit that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

If, at any time, it appears that a phone discussion would be helpful, the undersigned would greatly appreciate the opportunity to discuss such issues at the Examiner's convenience. The undersigned can be contacted at (617) 248-5000 or (617) 248-5071 (direct dial).

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Respectfully submitted,

A handwritten signature in cursive script, reading "Monica R. Gerber".

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